

Bioproduction of butanol from biomass: from genes to bioreactors

Thaddeus Chukwuemeka Ezeji¹, Nasib Qureshi² and Hans Peter Blaschek¹

Butanol is produced chemically using either the oxo process starting from propylene (with H₂ and CO over a rhodium catalyst) or the aldol process starting from acetaldehyde. The key problems associated with the bioproduction of butanol are the cost of substrate and butanol toxicity/inhibition of the fermenting microorganisms, resulting in a low butanol titer in the fermentation broth. Recent interest in the production of biobutanol from biomass has led to the re-examination of acetone-butanol-ethanol (ABE) fermentation, including strategies for reducing or eliminating butanol toxicity to the culture and for manipulating the culture to achieve better product specificity and yield. Advances in integrated fermentation and *in situ* product removal processes have resulted in a dramatic reduction of process streams, reduced butanol toxicity to the fermenting microorganisms, improved substrate utilization, and overall improved bioreactor performance.

Addresses

¹ University of Illinois, Biotechnology & Bioengineering Group, Department of Food Science & Human Nutrition, 1207 West Gregory Drive, Urbana, IL 61801, USA

² United States Department of Agriculture, National Center for Agricultural Utilization Research, Fermentation Biotechnology, 1815 North University Street, Peoria, IL 61604, USA

Corresponding author: Blaschek, Hans Peter (blaschek@uiuc.edu)

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Introduction

Anaerobic bacteria such as the solventogenic clostridia are capable of converting a wide range of carbon sources (e.g. glucose, galactose, cellobiose, mannose, xylose and arabinose) to fuels and chemicals such as butanol, acetone, and ethanol [1•]. However, butanol toxicity to the fermenting microorganisms limits its concentration in the fermentation broth, resulting in low butanol yields and a high cost for butanol recovery from the dilute solutions. During the past decade, the application of molecular techniques to the solventogenic clostridia — combined with recent advances in fermentation techniques — have resulted in the development of a hyper-butanol-producing strain and an

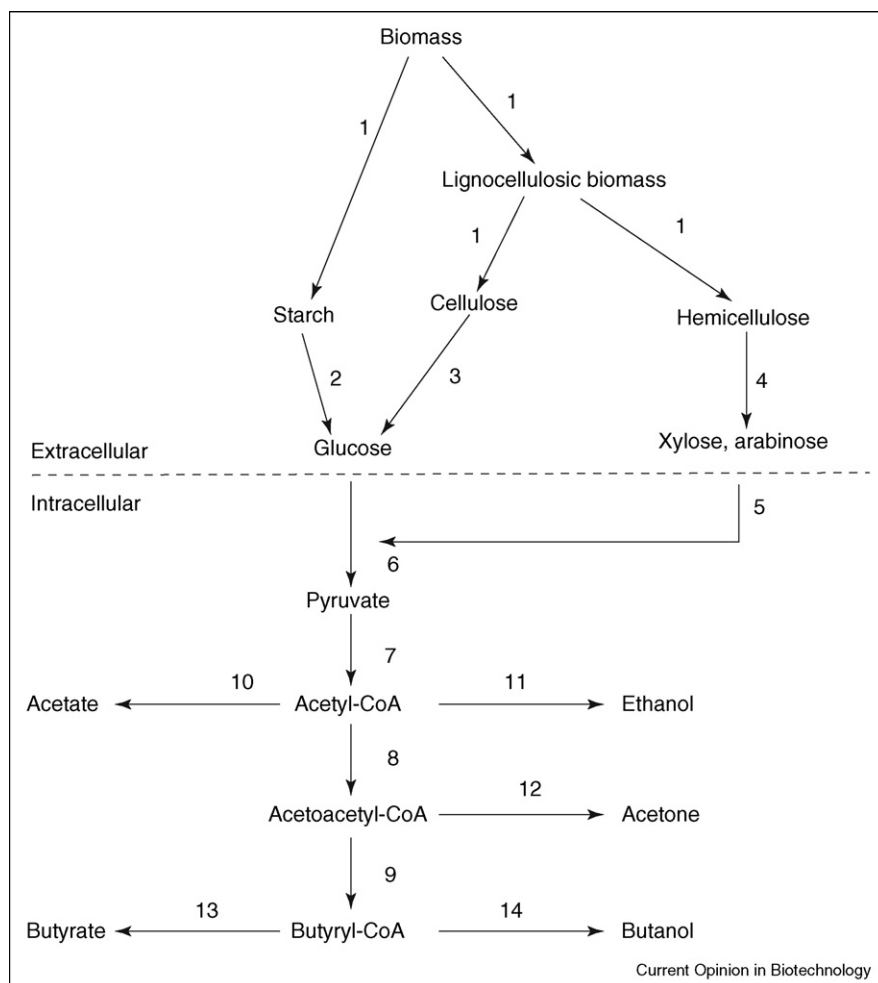
integrated acetone-butanol-ethanol (ABE) fermentation system for the simultaneous production and removal of butanol from the fermentation broth [2].

Efforts have been made to understand the mechanisms of sugar transport, regulation of butanol production, butanol tolerance, utilization of lignocellulosic biomass hydrolysates, and cell inhibition by lignocellulosic degradation products, with the aim of improving butanol productivity, titer and yield. Genetic manipulation of the microorganism depends on the development of effective gene transfer and associated systems. Genetic tools for transformation of the solventogenic clostridia have been developed and transformation of this genus with suitable genes coding for active hydrolytic enzymes would increase not only the utilization of a variety of carbon sources but also the efficiency. This review will focus on current advances in biobutanol production with reference to biomass utilization, strain improvement, bioreactor design and operations.

Regulation of carbohydrate utilization in solventogenic clostridia

The solventogenic clostridia have received much attention in recent years, because of their ability to produce industrially relevant chemicals such as butanol and 1,3-propanediol. The clostridia secrete numerous enzymes that facilitate the breakdown of polymeric carbohydrates into monomers (Figure 1). The secreted carbohydrate-degrading enzymes include, but are not limited to, α-amylase, α-glucosidase, β-amylase, β-glucosidase, glucoamylase, pullulanase, and amylopullulanase. The various monosaccharides produced are transported into the cell by specific membrane-bound transport systems, and the carbohydrates are subsequently metabolized via glycolysis or the pentose phosphate pathway (Figure 1). This ability to utilize mixed sugars is of particular relevance for the use of inexpensive agricultural by-products and wastes as fermentation substrates, because fermentation substrate is an important factor influencing the cost of biobutanol production [3]. Genomic sequence data of *Clostridium acetobutylicum* 824 demonstrated the presence of more than 90 genes encoding enzymes involved in the degradation of carbohydrate polymers, including at least 14 distinct families of glycosyl hydrolases [4]. Likewise, the draft genome sequence data for *Clostridium beijerinckii* 8052 recently made available by the Department of Energy (DOE) Joint Genome Institute suggested that *C. beijerinckii* 8052 has the genetic potential for utilization of a wide variety of carbohydrates (http://genome.ornl.gov/microbial/cbei/13may05/fc_Carbohydrate_Metabolism).

Figure 1



Simplified metabolism of biomass by solventogenic clostridia. 1, Pretreatment of corn and lignocellulose; 2, starch hydrolysis (α -amylase, β -amylase, pullulanase, glucoamylase, α -glucosidase); 3, cellulose hydrolysis (cellulases, β -glucosidase); 4, hemicellulose hydrolysis (see Figure 2); 5, xylose/arabinose uptake and subsequent breakdown via the transketolase-transaldolase sequence producing fructose 6-phosphate and glyceraldehydes 3-phosphate with subsequent metabolism by the Embden-Meyerhof-Parnas (EMP) pathway; 6, glucose uptake by the phosphotransferase system (PTS) and conversion to pyruvate by the EMP pathway; 7, pyruvate-ferrodoxin oxidoreductase; 8, thiolase; 9, 3-hydroxybutyryl-CoA dehydrogenase, crotonase and butyryl-CoA dehydrogenase; 10, phosphate acetyltransferase and acetate kinase; 11, acetaldehyde dehydrogenase and ethanol dehydrogenase; 12, acetoacetyl-CoA:acetate/butyrate:CoA transferase and acetoacetate decarboxylase; 13, phosphate butyltransferase and butyrate kinase; 14, butyraldehyde dehydrogenase and butanol dehydrogenase.

html). The genes that make up the carbohydrate catabolic operons are usually expressed when the respective substrates are present in the fermentation medium or when the preferred carbon sources are depleted (TC Ezeji and HP Blaschek, unpublished). Therefore, for efficient fermentation of lignocellulosic hydrolysates, it is imperative to heat shock (to induce expression of heat shock proteins that enhance butanol tolerance and production) the solventogenic clostridia in media containing the representative carbohydrates that are present in the fermentation medium [1^{••}]. So far, the solventogenic clostridia have not been shown to directly utilize crystalline cellulose or lignocellulosic biomass as a carbon source. Genome sequence analysis of *C. acetobutylicum* 824 revealed 11

proteins identified as cellulosome components. Ten of the genes encoding these components are organized in operon-like clusters (CAC910 to CAC919) with a gene order similar to that of *C. cellulolyticum* and *C. cellulovorans* [4]; nevertheless, *C. acetobutylicum* 824 lacks the ability to degrade crystalline cellulose. As part of an effort to induce the expression of cellulase-encoding genes in *C. acetobutylicum* 824, a certain level of cellulase secretion was induced by the utilization of xylose, lichenan, and cellobiose as fermentation substrates for butanol production [5]. By contrast, the current draft sequence of *C. beijerinckii* 8052 indicated an absence of native cellulosome-encoding genes. As a consequence, the ability of *C. beijerinckii* 8052 to degrade cellulose will have to be achieved through

the heterologous expression of introduced cellulosome genes from other species. Furthermore, although at least seven genes encoding xylan-degrading enzymes have been identified in *C. beijerinckii* 8052, recent investigations in our laboratory demonstrated that *C. beijerinckii* does not have the ability to degrade xylan or arabinoxylan for butanol production (TC Ezeji and HP Blaschek, unpublished).

Butanol fermentation by solventogenic clostridia

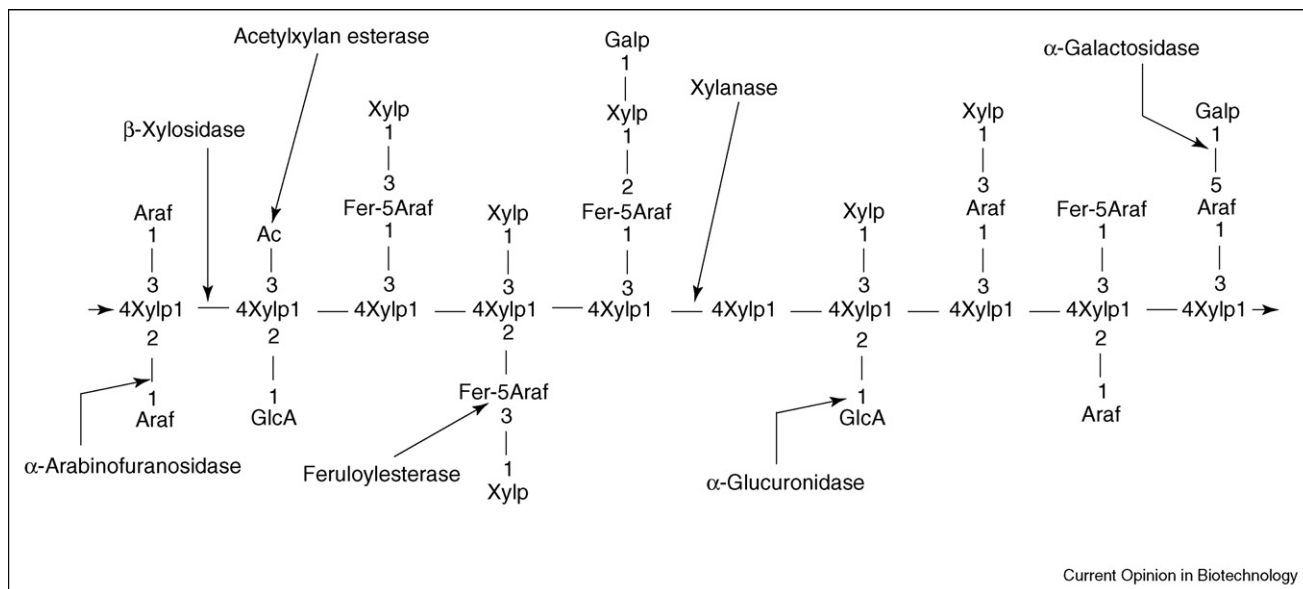
The fermentation profile of a microorganism is ultimately determined by its genetic makeup, which in turn controls the expression of relevant enzymes. An important advantage of the solventogenic clostridia is the variety of fermentation products (acetone, butanol, ethanol, acetic, butyric, lactic acids, etc.) that can be synthesized by this group of microorganisms. However, the loss of available carbon as a result of the formation of unwanted products is an undesirable property of the solventogenic clostridia. Clearly, enzyme synthesis and control of electron flow in the glycolytic pathway are vital with respect to the regulation of the butanol fermentation pathways. The presence of ferredoxin (Figure 1; 7) is common among the solventogenic clostridia and the direction of electron flow around reduced ferredoxin could have a crucial impact on the type and quantity of fermentation products produced. In addition, the ability of the solventogenic clostridia to grow under a low redox potential enables them to undertake a variety of stereospecific reductions, yielding chiral products that are difficult to synthesize chemically [6]. As the electron flow can be reversed, butanol yield should respond to factors that influence the direction of electron flow [7]. This observation has caused researchers to test the effect of numerous reducing compounds, such as carbon monoxide gassing, addition of methyl viologen, and the addition of neutral red into the fermentation medium during the ABE fermentation. In the presence of these electron carriers, butanol and ethanol formation were stimulated at the expense of acetone synthesis [7]. In addition, a completely different product, 1,3-propanediol, was synthesized by *C. acetobutylicum* 824 and *C. butyricum* VPI 3266 when glycerol, which is a more reduced substrate than glucose, was used as the carbon source [8]. Therefore, the development of an optimal fermentation medium and process for biobutanol production lies in our understanding of the physiology of the bacterium and associated critical interactions between carbon pathways and electron flow.

In the past few years, several carbon sources, such as glucose, starch, corn, molasses and soy molasses, have been utilized for laboratory biobutanol production. As substrate cost has a dramatic influence on butanol price [3], we have recently focused our research on the use of agricultural residues for biobutanol production when using *C. beijerinckii* BA101. In particular, we have focused on the use of corn stover, corn fiber, and fiber-rich distillers dried grains

and solubles (DDGS) as substrates for butanol fermentation. Although research on the genetics, fermentation, and downstream processing has progressed significantly in recent years, the solventogenic clostridia are not able to efficiently hydrolyze fiber-rich agricultural residues. For this reason, agricultural biomass must be pretreated and hydrolyzed to simple sugars using economical methods. Unfortunately, during pretreatment and hydrolysis, a complex mixture of microbial inhibitors is generated [1•]. Various pretreatment methods such as the use of dilute acid [9], hot water controlled pH [10], and ammonia fiber expansion (AFEX) [11•] are now available to solubilize and depolymerize biomass. Dilute acid pretreatment methods generate significant microbial inhibitors, while hot water and AFEX pretreatment methods generate only low concentrations of inhibitors. Complete hydrolysis of hemicellulose requires xylanase, β -xylosidase, and several other complimentary enzymes, such as acetylxyloxyesterase, α -arabinofuranosidase, α -glucuronidase, α -galactosidase, ferulic and/or *p*-coumaric acid esterase (Figure 2). The activities of these enzymes, in addition to the activities of cellulases on the cellulose component of the biomass, result in the generation of complex mixture of acids (e.g. ferulic, *p*-coumaric, acetic, glucuronic) in addition to monomeric sugars such as glucose, galactose, xylose, and arabinose in the biomass hydrolysates. Compounds such as ferulic and *p*-coumaric acids have been found to be inhibitory to the solventogenic clostridia at concentrations as low as 0.3 g/L [1•]. The undesirable lignocellulosic hydrolysate components can be divided into three groups on the basis of their origin: compounds released from the hemicellulose component (e.g. acetic, ferulic, glucuronic, *p*-coumaric acids, etc; Figure 2); lignin degradation products (e.g. syringaldehyde, syringic acid, etc); and sugar degradation products (e.g. furfural, hydroxymethyl furfural [HMF], and levulinic acid). Therefore, for complete depolymerization of lignocellulosic biomass, it is difficult to totally avoid the generation of inhibitory compounds irrespective of the pretreatment and hydrolysis method utilized.

Fermentation of this complex mixture of products by alcohol-producing microorganisms remains a challenge. Most bacteria utilize glucose as the preferred carbon source, and only when glucose is limiting are the pentose sugars utilized. From an economic point of view, it would be preferable for the pentose and hexose sugars to be used concurrently for butanol production, rather than sequentially. The solventogenic clostridia have been shown to utilize other sugars, including cellobiose, galactose, mannose, arabinose and xylose [1•] (TC Ezeji and HP Blaschek, unpublished). *C. beijerinckii* BA101 produced the highest concentration of butanol when cellobiose was used as the carbon source, whereas the least amount of butanol was produced when galactose was used [1•]. Sugar transport across the cell membrane is vital for efficient carbon utilization and butanol production. Solventogenic

Figure 2



Schematic depicting the basic structure of corn fiber heteroxylan and the enzymes needed to degrade the polymer to monomeric compounds. Araf, arabinofuranose; Xylp, xylopyranose; Galp, galactopyranose; GlcA, glucuronic acid; Fer, ferulic acid; AC, acetylxyylan (Figure adapted from [28].)

clostridia and many anaerobes transport sugars into the cell through the cell membrane using the phosphoenolpyruvate-dependent phosphotransferase system (PTS) [12], which is involved in the transfer of a phosphate group from phosphoenolpyruvate (PEP) to the sugar. Although glucose and fructose phosphorylation were supported by PEP, indicating the involvement of a PTS in the uptake of these sugars, galactose appears to be transported by a non-PTS mechanism, because a significant rate of phosphorylation of this sugar was supported by ATP rather than PEP [7]. However, *C. beijerinckii* BA101 and 8052 have been shown to involve both PTS and non-PTS transport systems simultaneously for the uptake of glucose during fermentation, with the non-PTS system (ATP-dependent glucokinase) predominant during the solventogenic phase when the PTS is repressed [12,13]. Therefore, it seems likely that galactose uptake occurs by a non-PTS mechanism and that its phosphorylation is catalyzed by galactokinase. Efficient galactose uptake in *C. beijerinckii* and fermentation to butanol might depend on the expression of galactokinase genes and the activity of galactokinase enzymes (ATP- α -D-galactose-1-phosphate transferase (galactokinase), uridine diphosphoglucose-D-galactose-1-phosphate uridylyl transferase, and uridine diphosphogalactose-4-epimerase) during the acidogenic and solventogenic growth phases. During the fermentation of mixed sugars with solventogenic clostridia (*C. beijerinckii* BA101, *C. acetobutylicum* 824, *C. beijerinckii* P-260, *C. butylicum* 592 and *C. saccharobutylicum* 292), all the sugars were utilized concurrently throughout the fermentation, although the rate of sugar utilization was sugar specific [1] (TC Ezeji and HP

Blaschek, unpublished). The concurrent uptake and metabolism of the hexose and pentose sugars is a desirable fermentation characteristic. It should be noted that in addition to being able to ferment both hexose and pentose sugars, the fermenting microorganisms must function in the presence of microbial inhibitors, especially those inhibitory compounds that are part of the hemicellulose structure (Figure 2). Adaptation of solventogenic clostridia in dilute acid hydrolyzed corn fiber medium (containing representative inhibitors) before fermentation has been shown to improve fermentation of lignocellulosic hydrolysates by more than 100% (TC Ezeji and HP Blaschek, unpublished).

Genetic strain improvement

The biobutanol fermentation suffers from several limitations (e.g. low titer, yield and productivity) and improvements in the performance of the solventogenic clostridia are necessary to move biobutanol fermentation research to a competitive commercial position. Several approaches have been employed to improve the performance of solventogenic clostridia with the aim of generating strains that can be used in industrial biobutanol production. Recombinant DNA technology, in addition to traditional mutagenesis and selection, have been employed to modify targeted metabolic pathways in the solventogenic clostridia.

Progress has been made over the past decade with respect to genetic manipulation of the solventogenic clostridia. The ability to induce mutagenesis in microorganisms and

to isolate mutants of interest has played a major role in the improvement of not only solventogenic clostridia but other industrially important microbes. Mutagens such as hydrogen peroxide, nalidixic acid, metronidazole, ethyl methanesulfonate, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, and UV irradiation have been used to induce mutations in solventogenic clostridia; amongst these, direct acting *N*-methyl-*N*-nitro-*N*-nitrosoguanidine appears to work best for the solventogenic clostridia. The mutant *C. beijerinckii* BA101, known for its stability and hyper-amyolytic and hyper-butanogenic characteristics, was generated in 1991 following treatment with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (reviewed by Ezeji *et al.* [2]). In 2003, an attempt was made to increase the butanol/acetone ratio in *C. acetobutylicum* 824 fermentation using antisense RNA strategies. Tummala *et al.* [14] performed several experiments using antisense RNA to downregulate the enzymes in the acetone formation pathway (acetoacetate decarboxylase [AADC]) and coenzyme A transferase [CoAT]). Despite significant downregulation of the CoAT gene and the production of substantially lower levels of acetone, there was no redirection of the carbon flux towards butanol synthesis; rather, the butanol level was drastically reduced in the resultant mutants. Another attempt was made to increase the butanol/acetone ratio through overexpression of the alcohol-aldehyde dehydrogenase (*aad*) gene and downregulation of CoAT using antisense RNA against *ctfB* (the second CoAT gene on the polycistronic *aad-ctfA-ctfB* message [15]). Again, the butanol level was drastically reduced in the resultant mutant, while the ethanol concentration was 23-fold higher than the control — the highest ethanol level ever reported in *C. acetobutylicum*. Although progress has been made, the use of recombinant DNA technology has so far not yielded a hyper-butanol-producing industrial strain, probably because of a lack of understanding of the global regulation of butanol production and the unique physiology of the solventogenic clostridia. As butanol is highly toxic to the solventogenic clostridia, metabolic engineering of various microorganisms not typically associated with butanol production, but resistant to butanol toxicity, has been suggested. In addition, simultaneous fermentation and recovery to relieve butanol toxicity (discussed later) might be the best short-term solution given the currently available solventogenic clostridia strains.

Advanced fermentation techniques and novel downstream processing

Traditionally, batch fermentations were commonly used for butanol production because suitable technologies to address the product toxicity problems associated with ABE fermentation were not available. During the 1940s and 1950s, biobutanol production on an industrial scale (Terre Haute, IN and Peoria, IL) was carried out using large batch fermentors ranging in capacity from 200 000 to 800 000 L. The industrial process used 8–10%

corn mash, which was cooked for 90 min at 130–133 °C. Sugar cane molasses was also used to produce biobutanol in a commercial plant in South Africa until the early 1980s. It should be noted that at a concentration of approximately 16 g butanol/L, cell growth inhibition and premature termination of the fermentation occurs. Product toxicity results in low butanol concentration in the reactor. In addition, the use of a dilute sugar solution results in large process volumes. Because of these problems, the production of biobutanol on a commercial scale has been considered to be uneconomical. Various substrates can be used to produce butanol including corn (dry grind and wet milling processes), molasses, whey permeate or glucose derived from corn [16]. Biobutanol production is a biphasic fermentation where acetic and butyric acids are produced during the acidogenic phase followed by their conversion into acetone and butanol (solventogenic phase). At the end of the fermentation, cell mass and other suspended solids are removed by centrifugation and can be sold as cattle feed. In several recent approaches, agricultural waste such as packing peanuts (peanut-shaped starch-based packing material), orchard waste [16], DDGS (TC Ezeji, HP Blaschek, unpublished), corn fiber [17], and wheat straw [18••] have been used as substrates.

During the past two decades a significant amount of research has been performed on the use of alternative fermentation and product recovery techniques for biobutanol production. These techniques have involved the use of immobilized and cell recycle continuous bioreactors and alternative product recovery technique (e.g. adsorption, gas stripping, ionic liquids, liquid–liquid extraction, pervaporation, aqueous two-phase separation, supercritical extraction, and perstraction, etc). The application of some of these techniques to the ABE fermentation process is described below.

Immobilized and cell recycle continuous bioreactors

In a biobutanol batch process, reactor productivity is limited to less than 0.50 g/L/h for a number of reasons, including low cell concentration, down time, and product inhibition (reviewed in [19]). In a batch reactor a cell concentration of <4 g/L is normally achieved. The cell concentration inside the bioreactor can be increased by one of two techniques, namely ‘immobilization’ or ‘cell recycle’. In a study to explore different cell supports (e.g. clay brick) for *C. beijerinckii* cells, Qureshi *et al.* [16] were able to improve reactor productivity to 15.8 g/L/h. In another approach, Huang *et al.* [20] immobilized cells of *C. acetobutylicum* in a fibrous support and used these in a continuous reactor to produce ABE; a productivity of 4.6 g/L/h was obtained. Cell recycle — where cells are returned to the bioreactor using a filter and clear liquid is removed — can also be used to increase cell concentration in the reactor and to improve reactor productivity. Using

this approach, reactor productivities up to 6.5 g/L/h (as compared to <0.5 g/L/h in batch fermentation) were achieved in biobutanol fermentation (reviewed in [19]).

Gas stripping

Gas stripping is a technique that can be applied for *in situ* butanol recovery during the ABE fermentation [19,21,22]. The production of ABE is associated with the generation of gases (CO₂ and H₂). In an attempt to make the process of ABE recovery from the fermentation broth simpler and more economical, these fermentation gases are used to recover butanol during simultaneous fermentation and *in situ* recovery by gas stripping [19,21,22]. The gases are bubbled through the fermentation broth and then cooled in a condenser. As the gas is bubbled through the fermentor, it captures ABE which is subsequently condensed and collected in a receiver vessel. Once the solvents are condensed, the gas is recycled back to the fermentor to capture more ABE. This process continues until all the sugar in the fermentor is utilized by the culture or the fermentation is terminated. In some cases a separate stripper can be used to strip off solvents, the stripper effluent is then recycled back to the reactor [21]. A schematic diagram of ABE production and recovery by gas stripping has been published elsewhere [23,24]. Table 1 compares some of the selected fermentation parameters obtained in non-integrated and integrated (with product recovery) batch, fed-batch, and continuous systems.

Liquid–liquid extraction

The removal of butanol or ABE from fermentation broth by liquid–liquid extraction is considered an important technique. Usually, a water-insoluble organic extractant is mixed with the fermentation broth. Butanol is more soluble in the organic (extractant) phase than in the aqueous (fermentation broth) phase; therefore, butanol selectively concentrates in the organic phase. As the extractant and fermentation broth are immiscible, the extractant can easily be separated from the fermentation broth after butanol extraction. It should be noted that

liquid–liquid extraction is able to extract butanol from the fermentation broth without removing substrates, water or nutrients. The extractant of choice among researchers has been oleyl alcohol because it is relatively non-toxic, as well as being a good extractant [19,25].

Perstraction

Several problems are associated with liquid–liquid extraction, such as toxicity to the cells, the formation of an emulsion, loss of extraction solvent, and the accumulation of microbial cells at the extractant and fermentation broth interphase. To solve these concerns, a new technique called ‘perstraction’ was developed [19]. In a perstractive separation, the fermentation broth and the extractant are separated by a membrane. The membrane contactor provides surface area where the two immiscible phases can exchange butanol. As there is no direct contact between the two phases, extractant toxicity, phase dispersion, emulsion and rag layer formation (i.e. the accumulation of cells at the aqueous–organic interphase) are drastically reduced or eliminated. In such a system, butanol would diffuse preferentially across the membrane, while other components and fermentation intermediates (e.g. acetic and butyric acids) are retained in the aqueous phase [26]. The total mass transport of butanol from the fermentation broth to the organic side depends on the rate of diffusion of butanol across the membrane. The membrane does, however, present a physical barrier that can limit the rate of butanol extraction.

Pervaporation

Pervaporation is a technique that allows the selective removal of volatile compounds from model solution/fermentation broth using a membrane. The membrane is placed in contact with the fermentation broth and the volatile or organic component selectively diffuses through the membrane as a vapour. The compound is then recovered by condensation. In this process, a phase change occurs from liquid to vapor. As it is a selective removal process, the desired component requires a heat of vaporization at the feed temperature. The mechanism by

Table 1

A comparison of novel ABE production systems using glucose as substrate.

| Fermentation process | Culture | Sugar used g/L ^a | Total ABE produced g/L | Yield | ABE productivity (g/L/h) | Ref |
|---|------------------------------|-----------------------------|------------------------|-------------------|--------------------------|------|
| Batch (control) | <i>C. beijerinckii</i> BA101 | <60 | <33 | 0.38–0.40 | 0.35 | [19] |
| Product recovery by gas stripping: | | | | | | |
| Batch | <i>C. beijerinckii</i> BA101 | 161 | 75.9 | 0.47 ^b | 0.61 | [23] |
| Fed-batch | <i>C. beijerinckii</i> BA101 | 500 | 233 | 0.47 ^b | 1.16 | [24] |
| Continuous | <i>C. beijerinckii</i> BA101 | 1163 | 460 | 0.40 | 0.91 | [22] |
| Product recovery by pervaporation: | | | | | | |
| Fed-batch | <i>C. acetobutylicum</i> 824 | 470 ^c | 155 | 0.31–0.35 | 0.13–0.26 | [19] |

^a g per L culture volume.

^b values are higher than expected.

^c calculated value.

which a volatile/organic component is removed by pervaporation is called solution-diffusion. The effectiveness of pervaporation is measured by two parameters: the selectivity (a measure of the selective removal of volatiles) and flux (the rate at which an organic/volatile passes through the membrane per m² membrane area). The details of pervaporation have been reviewed by Qureshi and Blaschek [16] and by Ezeji *et al.* [19].

Economics of the ABE fermentation

In recent years several economic studies have been performed on the production of butanol from corn (dry corn and wet corn milling) whey permeate, and molasses [19,21]. In these studies it was determined that the distillative recovery of biobutanol from the fermentation broth is not economical when compared with butanol derived from the current petrochemical route. Nevertheless, studies employing *C. beijerinckii* BA101, *C. acetobutylicum* P260, hydrolyzed DDGS and wheat straw suggest that commercial production of biobutanol from agricultural byproducts/wastes is drawing closer. Recently, DuPont (US) and BP (UK) announced their plans to invest in biobutanol production research. It is anticipated that the first plants would operate on sugar or corn starch; however, it is likely that agricultural waste would become a potential substrate in the near future.

Several recent advances have been made including the development of microbial cultures, process technologies, and use of waste substrates; however, these advances will need to be further developed to run a fermentation-based biobutanol industry that can compete effectively with petrochemically derived butanol. It is suggested that future research might focus on the development of second-generation cultures (as compared to the existing strains *C. beijerinckii* BA101, *C. acetobutylicum* PJC4BK, and *C. acetobutylicum* P260, which produce total ABE on the order of 25–33 g/L [16,19]). Another avenue where technological advances could be made involves the recovery of fermentation by-products (large waste water streams, cell mass, CO₂, and H₂) for further revenue. For example, CO₂ can be converted to algal biomass and oil in the presence of sunlight; use of this zero cost substrate (CO₂) would benefit the biobutanol industry significantly. Additionally, H₂ gas can be separated and used as an excellent source of energy.

Conclusions

The use of lignocellulosic biomass as a substrate for biobutanol fermentation has great potential. Much progress has been made with the use of DDGS (TC Ezeji, HP Blaschek, unpublished), wheat straw [18^{••}], and corn fiber xylan [27^{••}]. Nevertheless, to use substrates such as corn fiber hydrolysate and to meet the economic challenges associated with this fermentation, new strains that are both capable of utilizing mixtures of lignocellulosic-derived sugars and resistant to microbial inhibitors

present in the lignocellulosic hydrolysate need to be developed. Alternately, economic methods for the removal of inhibitors from these hydrolysates could also be developed. Biobutanol fermentation technology has been changing at a rapid pace. In the authors' opinion, the production of biobutanol using biomass is getting closer to scale-up and possible commercialization. The use of lignocellulosic substrates in combination with developed process technology is expected to make the production of biobutanol economically viable.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Ezeji TC, Qureshi N, Blaschek HP: **Butanol production from agricultural residues: impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation.** *Biotechnol Bioeng* 2007. [Epub ahead of print: DOI:10.1002/bit.21373].
- This research article demonstrates the effect of compounds such as salts, furfural, hydroxymethyl furfural (HMF), acids (acetic, ferulic, glucuronic, *p*-coumaric), and phenolic compounds, which are potential degradation products generated during lignocellulosic biomass pretreatment and hydrolysis on cell growth and butanol production. Some of these inhibitory products negatively affect cell growth and butanol production by *Clostridium beijerinckii*. Furfural and HMF are not inhibitory to *C. beijerinckii*; rather they have a stimulatory effect on the growth of the microorganism and ABE production.
2. Ezeji TC, Qureshi N, Blaschek HP: **Butanol fermentation research: upstream and downstream manipulations.** *Chem Rec* 2004, 4:305-314.
3. Qureshi N, Blaschek HP: **Economics of butanol fermentation using hyper-butanol producing *Clostridium beijerinckii* BA101.** *Trans IChemE* 2000, 78(Part C):139-144.
4. Nolling J, Breton G, Omelchenko MV, Makarova KS, Zeng Q, Gibson R, Lee HM, Dubois J, Qiu D, Hitti J *et al.*: **Genome sequence and comparative analysis of the solvent-producing bacterium *Clostridium acetobutylicum*.** *J Bacteriol* 2001, 183:4823-4838.
5. Lopez-Contreras AM, Gabor K, Martens AM, Renckens BAM, Claassen PAM, van der Oost J, Willem M, de Vos WM: **Substrate-induced production and secretion of cellulases by *Clostridium acetobutylicum*.** *Appl Environ Microbiol* 2004, 70:5238-5243.
6. Tomas CA, Tummala SB, Papoutsakis ET: **Metabolic engineering of solventogenic clostridia.** In *Handbook on Clostridia*. Edited by Durre P. Boca Raton, Florida: CRC Press, Taylor & Francis Group; 2005:813-830.
7. Mitchell WJ: **Physiology of carbohydrates to solvent conversion by clostridia.** *Adv Microb Physiol* 1998, 39:31-130.
8. González-Pajuelo M, Meynial-Salles I, Filipa Mendes F, Soucaille P, Vasconcelos I: **Microbial conversion of glycerol to 1,3-propanediol: physiological comparison of a natural producer, *Clostridium butyricum* VPI 3266, and an engineered strain, *Clostridium acetobutylicum* DG1(pSPD5).** *Appl Environ Microbiol* 2006, 72:96-101.
9. Purwadi R, Niklasson C, Taherzadeh MJ: **Kinetic study of detoxification of dilute acid hydrolysates by Ca(OH)₂.** *J Biotechnol* 2004, 114:187-198.

10. Mosier N, Hendrickson R, Ho N, Sedlak M, Ladisch MR: **Optimization of pH controlled liquid hot water pretreatment of corn stover.** *Bioresour Technol* 2005, **96**:1986-1993.
 11. Teymouri F, Laureano-Perez L, Alizadeh H, Dale BE: **Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover.** *Bioresour Technol* 2005, **96**:2014-2018.
- This article demonstrates that a mild pretreatment method such as AFEX greatly enhances the digestibility of biomass and that high glucose and xylose yields are achievable even at low enzyme levels.
12. Lee J, Blaschek HP: **Glucose uptake in *Clostridium beijerinckii* NCIMB 8052 and the solvent-hyperproducing mutant BA101.** *Appl Environ Microbiol* 2001, **67**:5025-5031.
 13. Lee J, Mitchell WJ, Tangney M, Blaschek HP: **Evidence for the presence of alternative glucose transport system in *Clostridium beijerinckii* NCIMB 8052 and the solvent-hyperproducing mutant BA101.** *Appl Environ Microbiol* 2005, **71**:3384-3387.
- This study demonstrates that wild-type *C. beijerinckii* NCIMB 8052 and the mutant strain *C. beijerinckii* BA101 both have a PTS and a non-PTS uptake system for glucose, with their relative contributions to glucose accumulation being dependent on the physiological state of the cells.
14. Tummala SB, Welker NE, Papoutsakis ET: **Design of antisense RNA constructs for downregulation of the acetone formation pathway of *Clostridium acetobutylicum*.** *J Bacteriol* 2003, **185**:1923-1934.
 15. Tummala SB, Junne SG, Papoutsakis ET: **Antisense RNA downregulation of coenzyme A transferase combined with alcohol-aldehyde dehydrogenase overexpression leads to predominantly alchologenic *Clostridium acetobutylicum* fermentations.** *J Bacteriol* 2003, **185**:3644-3653.
 16. Qureshi N, Blaschek HP: **Butanol production from agricultural biomass.** In *Food Biotechnology*. Edited by Shetty K, Pometto A, Paliyath G. Boca Raton, FL: Taylor & Francis Group plc; 2005:525-551.
 17. Qureshi N, Ebener J, Ezeji TC, Dien B, Cotta MA, Blaschek HP: **Butanol production by *Clostridium beijerinckii* BA101. Part I: Use of acid and enzyme hydrolysed corn fiber.** *Bioresour Technol* 2007: in press.
 18. Qureshi N, Saha BC, Hughes SR, Cotta MA: **Production of acetone butanol (AB) from agricultural residues using *Clostridium acetobutylicum* in batch reactors coupled with product recovery.** *Ninth International Workshop and Conference on the Regulation of Metabolism, Genetics and Development of the Solvent and Acid Forming Clostridia*. Rice University, Houston, May 18-21, 2006.
- This presentation demonstrates that acetone butanol can be produced from hydrolysates of agricultural residues such as wheat straw. Using wheat straw hydrolysate (WSH), ABE productivity was improved by 214%. WSH supplemented with sugar was successfully fermented when coupled with a novel product recovery technique.
19. Ezeji TC, Qureshi N, Karcher P, Blaschek HP: **Butanol production from corn.** In *Alcoholic Fuels: Fuels for Today and Tomorrow*. Edited by Minteer SD. New York, NY: Taylor & Francis; 2006:99-122.
 20. Huang W-C, Ramey DE, Yang S-T: **Continuous production of butanol by *Clostridium acetobutylicum* immobilized in a fibrous bed reactor.** *Appl Biochem Biotechnol* 2004, **113**:887-898.
 21. Ezeji TC, Qureshi N, Blaschek HP: **Industrially relevant fermentations.** In *Handbook on Clostridia*. Edited by Durre P. Boca Raton, Florida: CRC Press, Taylor & Francis Group; 2005:797-812.
 22. Ezeji TC, Qureshi N, Blaschek HP: **Process for continuous solvent production.** U.S. Provisional Patent 2005, No. 60/504,280.
 23. Ezeji TC, Qureshi N, Blaschek HP: **Production of butanol by *Clostridium beijerinckii* BA101 and in-situ recovery by gas stripping.** *World J Microbiol Biotechnol* 2003, **19**:595-603.
 24. Ezeji TC, Qureshi N, Blaschek HP: **Acetone-butanol-ethanol production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping.** *Appl Microbiol Biotechnol* 2004, **63**:653-658.
 25. Karcher P, Ezeji TC, Qureshi N, Blaschek HP: **Microbial production of butanol: product recovery by extraction.** In *Microbial Diversity: Current Perspectives and Potential Applications*. Edited by Satyanarayana T, Johri BN. New Delhi: IK International Publishing House Pvt. Ltd; 2005:865-880.
 26. Qureshi N, Maddox IS: **Reduction in butanol inhibition by perstraction: Utilization of concentrated lactose/whey permeate by *Clostridium acetobutylicum* to enhance butanol fermentation economics.** *Official Journal of the European Federation of Chemical Engineering* (formerly Trans IChemE; Chemical Engineering Research & Design): Food and Bioproducts Processing. *Part C* 2005, **83**(C1):43-52.
 27. Qureshi N, Li X-L, Hughes S, Saha BC, Cotta MA: **Butanol production from corn fiber xylan using *Clostridium acetobutylicum*.** *Biotechnol Prog* 2006, **22**:673-680.
- Demonstrates that the integration of hydrolysis of corn fiber arabinoxylan, fermentation to ABE, and recovery of ABE in a single system is possible and economical. The paper is a definitive demonstration of the potential of ABE production from a biomass-based substrate.
28. Gray KA, Zhao L, Emptage M: **Bioethanol.** *Curr Opin Chem Biol* 2006, **10**:141-146.